U.S. ARMY MEDICAL RESEARCH
INSTITUTE OF CHEMICAL DEFENSE

USAMRICD-TR-91-06

DEVELOPMENT OF A SCREENING PROCEDURE FOR USE IN THE EVALUATION OF TOPICAL PROTECTANTS AFTER CHALLENGE WITH VESICATING AGENTS

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April 1991

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) USAMRICD-TR-91-06	5 MONITORING ORGANIZATION REPORT NUMBER(S) USAMRICD-TR-91-06
6a. NAME OF PERFORMING ORGANIZATION U.S. Army Medical Research Institute of Chemical Defense 6b. OFFICE SYMBOL (If applicable) SGRD-UV-DB	7a NAME OF MONITORING ORGANIZATION U.S. Army Medical Research Institute of Chemical Defense, SGRD-UV-RC
6c. ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground, MD 21010-5425	7b ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground, MD 21010-5425
8a NAME OF FUNDING/SPONSORING ORGANIZATION 8b OFFICE SYMBOL (If applicable)	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code)	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 62787A; PROJECT NO: 3M162787A; TASK NO: 875 BA; WORK UNIT ACCESSION NO:

11 TITLE (Include Security Classification)
Development of a Screening Procedure for use in the Evaluation of Topical Protectants after Challenge with Vesicating Agents

12 PERSONAL AUTHOR(S)
Forster, Jeffry S., Jacinto, Brenda, Hammond, Philip S., Gadson, Curtis, and Mershon, Millard M.

13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Technical	FROM _____ TO _____	April 1991	20

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	chlorovinyldichloro arsine, mustard, Lewisite, topical protectants, screening procedure
07	03		
07	04		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

1,1' -Thiobis[2-cloroethane] (bis(2-chloroethyl)sulfide, sulfur mustard, H) causes skin, respiratory and eye injury and has been identified as being mutagenic and carcinogenic. The development of topical protectants that can decrease the effects of cutaneous exposure to vesicating chemical warfare agents or that may enhance the effectiveness of decontamination through limiting penetration to the skin should significantly enhance a soldier's ability to carry out his or her job in a chemically hostile environment. Preliminary in vivo studies with a small number of candidate topical protectants demonstrated that they could provide protection against several different chemical agents.

Development of an in vivo procedure for evaluation of candidate topical protectants, one that would be predictive of in vivo results, would be of considerable advantage for the screening of these materials. A screening procedure would not only limit the

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	21 ABSTRACT SECURITY CLASSIFICATION
22a. NAME OF RESPONSIBLE INDIVIDUAL Stewart, James R., LTC, VC	22b TELEPHONE (Include Area Code) 301-671-4442 22c OFFICE SYMBOL SGRD-UV-D

DD Form 1473, JUN 86 *Previous editions are obsolete* SECURITY CLASSIFICATION OF THIS PAGE

19. cont'd

number of animals needed to evaluate these compounds, but also could provide results more rapidly than the *in vivo* procedure. In attempts to develop such a screen, one-inch holes were punched in plastic sheets of known thicknesses and were attached to M8 chemical detector paper. Candidate protectant formulations were spread in the resulting well and over the M8 chemical detector paper. The topical protectant layers were then challenged with either 2-chloroethyl ethyl-sulfide (CEES) or distilled bis(2-chloroethyl)sulfide (HD) by application to the top of the protectant. Time to penetration, as evidenced by a color change of the M8 chemical agent paper, was recorded for a series of these assemblies. Candidate protectant formulations performed with various levels of success, with several of the protectants maintaining their integrity against the agent challenge for at least 60 minutes, the time at which the tests were terminated.

PREFACE

The need for *in vitro* test procedures for the evaluation of candidate topical protectants was recognized by the Antivesicant Decision Tree Network Subcommittee of the Drug Assessment Technical Evaluation Committee. The work described is authorized under the US Army Medical Research Institute of Chemical Defense (USAMRICD) protocol number 1-11-89-000-A-535, "Development of an M8 Chemical Agent Paper Based Screening Procedure for Use in the Evaluation of Topical Protectants after Challenge with Vesicating Agents." The experimental data, results and conclusions have been recorded in notebook number 050-89.

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INTRODUCTION

1,1'-Thiobis[2-chloroethane] (bis(2-chloroethyl)sulfide, sulfur mustard, H) causes skin,[1,2] respiratory and eye injury and has been identified as being mutagenic[3] and carcinogenic.[4,5] The mechanism of action of H is still under investigation, as is the development of pretreatment compounds that can decrease the effects of cutaneous (or other) exposures of this compound to humans. There are no currently fielded therapeutics for vesicating agents. In addition, exposure to H or to another vesicating agent, chlorovinyldichloroarsine (Lewisite, L), causes a rapid toxic insult to the skin, leading to severe cutaneous damage. It has been demonstrated that these effects are largely irreversible when decontamination is delayed even by five minutes for exposures to H and almost immediately for exposures to L.[6]

One means by which this problem may be lessened involves an approach that can also reduce the risk of percutaneous exposure to organophosphorus chemical agents, i.e., using topical protectants as barriers to prevent skin penetration. Early evaluations of this approach, which examined commercially available barrier creams, concluded that the approach provided only minimal protection against organophosphorus compounds.[7] Later studies, however, using both in vitro and in vivo testing methods showed significant reduction in penetration or toxicity for several different chemical warfare agents.[8,9,10] One problem that was identified in these latter studies was that universal effectiveness was often not achieved.[10]

In light of the limited time period for effective decontamination following H exposure, it appeared that the use of an effective topical protectant against H would present a viable means of limiting the hazards of cutaneous exposure for this agent. Such a topical protectant, one that could decrease the effects of cutaneous exposure to vesicating chemical warfare agents or that could enhance the effectiveness of decontamination, should significantly enhance a soldier's ability to carry out his or her job in a chemically hostile environment.

A number of both in vitro and in vivo testing methods have been used to evaluate dermal toxicity following exposure to vesicating chemical agents. These methods, however, are too complicated to use in the early stages of a screening procedure, particularly if a large number of topical protectant samples are to be evaluated. The studies described in this report have focused on development of a testing screen to assist in the evaluation of topical protectants. Tests were carried out by first spreading a candidate topical protectant over chemical agent detection paper, followed by challenge with either simulant or the actual chemical agent. The depth of the protectant is controlled by a polyethylene sheet into which holes have been punched, creating a well for the candidate protectant. Mustard or simulant was applied to the surface of the protectant, with the test terminating when the agent or simulant penetrates the protectant causing the chemical agent detection paper to turn color, or after 60 minutes from the start of the test in cases where no penetration occurs. While penetration times could be compared for the various topical protectants, protectants that blocked breakthrough for less than 60 minutes gave results with substantial variance

within groups. Thus, while it may be difficult to quantitatively rank order topical protectants by this procedure, a qualitative sense of their performance is provided.

MATERIALS

2-(Chloroethyl) ethylsulfide (CEES) was obtained from Aldrich Chemical Company, Inc., 1,1'-thiobis[2-chloroethane] (bis(2-chloroethyl)sulfide, sulfur mustard, H) was obtained from the Chemical Research, Development and Engineering Center's (CRDEC) Research Laboratory. Mustard (H) had been further purified by CRDEC through distillation, and was subsequently designated HD for distilled mustard. The identity and HD content was assayed by NMR spectroscopy, and the HD was found to be of 97.9% purity. M8 Chemical Agent Detection paper (Stock number NSN 6665-00-050-8529) was obtained from Anachemia Canada, Inc., Lachine, Que., Canada; polyethylene sheets for spacer use were obtained from Almac Plastics Inc., 6311 Erdman Ave., Baltimore, MD 21205 and were of a 0.15 mm thickness. Polyethylene glycol 540 (PEG 540) was available from Union Carbide Co. Candidate topical protectants were obtained from the following commercial sources: E.I. Du Pont De Nemours & Company (inc.), Chemicals and Pigments Department, Jackson Laboratory, P.O. Box 525, Wilmington, Delaware 19899; Ausimont U.S.A., Inc., 44 Whippany Road, Morristown, NJ 07962-1838; Interpro, Inc., P.O. Box 1823, Haverhill, Ma 01831; Biocontrol, Inc., 8960 Springbrook Drive, Suite 105, Coon Rapids, MN 55433; Biomedic, Milan, Italy; Mane Street, Inc., Minneapolis, MN.

EXPERIMENTAL PROCEDURE

Test assemblies were prepared using polyethylene sheets of 0.15 mm thickness into which 1" diameter holes had been punched. Using an arch punch and a steel template for positioning, two rows of eight holes were punched into 5" X 12" cm^2 sheets of polyethylene. The polymer sheets were then coated with glue and attached to M8 paper, where the holes served as wells for the topical protectants. The wells were filled with excess candidate topical protectant and leveled by drawing a microscope slide over the plastic surface. This process served to both remove excess protectant and also to smooth the layer to provide an even challenge surface. Special care in this process was required to assure both the plastic sheet was flat against the M-8 paper, and that the protectant was smoothed evenly to a constant thickness. A stereoscopic microscope was used to evaluate the spreading of the protectant to make sure that there were no bubbles or troughs. Such areas could provide areas of lower thickness and allow a point penetration to occur. The filled wells were then provided with a "dam" of the same topical protectant of approximately 0.5 inches in diameter. This provided a centered cavity on the protectant to receive the sample of vesicant. In cases where the topical protectant material was too viscous, PEG 540 was used. The dam was used to control spreading of the vesicant challenge, which in some cases would travel to the edge of the protectant and then travel down to the M8 paper, causing a false breakthrough. Prior to exposure of the protectant layer to agent or simulant, the entire assembly was placed on a viewing device. That device consisted of a metal plate in which two rows of eight one inch holes had been punched. The plate was supported by metal legs approximately 10 inches long with a mirror attached between the supports for viewing of the underside of

the M-8 assemblies. All studies were conducted in an approved toxic chemical agent use hood, with face velocity of 100 ± 20 fpm. When working with the vesicating agents CEES and HD, the operator wore one pair of surgical gloves covered with a pair of chemical agent resistant gloves during the entire operation. These gloves were changed periodically, or when any chance of contamination was suspected

Mustard was received in a 2.0 ml crimp capped vial with a rubber septum. A 10 μl Hamilton syringe was used to withdraw samples from the agent vial. The syringe was withdrawn from the vial and moved to a location directly above the protective layer assembly. The entire dose of HD was expelled onto the protectant surfaces inside the dam. Challenges were made at an interval of every 30 seconds for HD and every 10 seconds for CEES. Challenges with the latter compound could be made more rapidly since the CEES was pipeted from an open container with a Gilson pipetor. Following completion of all challenges, a plastic cover was placed over the entire set of test assemblies, to limit evaporation of the agent or simulant. While many of the candidate topical protectants were transparent, so that breakthrough could be observed from either the top or the bottom of the holder, a few of the topical protectants were opaque, so observations of penetration times were made by observing the underside of the M-8 assembly via the mirror. Breakthrough times were recorded as the time when the M-8 paper turned red as it came in contact with mustard or CEES. The challenges were terminated at 60 minutes or when a breakthrough was recorded. All pieces of equipment that could have been contaminated by the mustard or simulant were decontaminated with 5% aqueous sodium hypochlorite or a mixture of High Test Hypochlorite (HTH) and aqueous 5% NaOCL.

RESULTS AND DISCUSSION

During development of this method, early experiments examined various methods of application for the topical protectants, various configurations of the assemblies, amounts of simulant used to challenge the topical protectants, etc. These studies were carried out using PEG 540 as a positive control, i.e., no breakthrough in at least 60 minutes, petrolatum as a barrier with intermediate breakthrough times, and the partially hydrogenated vegetable oil "Crisco"™ as the negative control, i.e., rapid breakthrough. From these studies, examining the breakthrough of methyl salicylate as a relatively non-toxic simulant, the following observations were made.

(1) While breakthrough times were relatively constant, variance in these values made use of the test procedure difficult for obtaining quantitative results. As an example, Table 1 presents three sets of data for layers of the same topical protectant and at the same thickness that were challenged with simulant. One may note that the average breakthrough time shows some variance from group-to-group, and that the mean values for breakthrough show large standard deviations.

Table 1: Breakthrough Times for 0.15 mm Layers of Crisco™ Challenged with 8 μl Chloroethyl Ethyl Sulfide (CEES)

Trial Number	Pos. No. 1	2	3	4	5	6	7	8	Breakthrough Times (sec) Mean (SD)
1	08	29	34	24	08	34	07	09	19 (12)
2	43	27	27	35	30	20	59	105	43 (28)
3	11	10	20	06	29	10	10	05	13 (08)

(2) One or more assemblies in a set often gave rapid breakthrough while the remainder could show no breakthrough, even at 60 minutes when observation was terminated. Examination of the layers under a stereoscopic microscope showed that, in particular for the PEG 540, many bubbles were trapped in the layer. It seems likely that these bubbles provide a path of little resistance for breakthrough in some cases, thus causing the rapid penetration of the simulant. Application of the layer while using a stereoscopic microscope to observe the procedure allowed for elimination of most of the bubbles, improved reproducibility of breakthrough times, and eliminated many of the rapid breakthroughs in these cases.

(3) Some variance was observed in time-to-penetration for a given layer, depending on the operator that applied the layer. Thus, when Crisco™ was tested on two separate days with the layer applied by either the same operator or by a different operator, the results showed a statistically significant change from operator-to-operator, but not from day-to-day (see Table 2).

While this could be minimized by practice, it seemed necessary to keep the same applicator from day-to-day, and this was typically the case.

Table 2: Time-to-Breakthrough Dependency on Applicator Identity Following a 8 μl Challenge of a Crisco™ Layer with 2-(Chloroethyl)ethyl sulfide (CEES)

Date	Applicator	Time (sec)	SD
09/18/89	1	165	51
	2	177	34
10/10/89	1	178	57
	3	254	37
	2	146	66
	3	227	70

*Results of an analysis of variance evaluation of these data indicate the following: applicators 1 and 2 show relatively reproducible results, both from applicator-to-applicator and from day-to-day; applications of the topical protectant by operator 3 gave breakthrough times that were consistent for that person, but that were statistically different from the other two applicators.

(4) Use of a dam to limit run-off of the applied liquid challenge was critical. If the candidate layer's surface was wet by the agent or simulant, lateral travel of the liquid was not a serious problem. However, in those cases where the simulant or agent did not wet the surface, very rapid exposure of the M8 paper test surface occurred through migration of the liquid to the interphase between the topical protectant and the polyethylene sheet. Use of a dam of the candidate topical protectant was effective at minimizing this problem.

Several other issues proved to be of particular importance in assuring that results were as reproducible as possible from day-to-day or group-to-group of assemblies. Special care is needed in the fabrication of the test assemblies to assure that the polyethylene spacing sheet firmly adheres to the M-8 paper. As noted previously, spreading the candidate topical protectant in a manner that eliminates air bubbles and troughs, and that provides a layer of uniform thickness throughout the test area, was of critical importance.

Compounds and materials examined as candidate topical protectants in these studies included representatives from three basic groups. The first were perfluorinated polyalkylethers of the types shown in structures 1-3, either examined by themselves or as thickened creams. Thickening was achieved by addition of materials such as powdered polytetrafluoroethylene (Teflon™, 4) and a fumed silica, Cab-O-Sil. The second group of materials examined as

candidate topical protectants included formulations available from commercial suppliers and specifically designed as barriers. The third group examined as candidate protectants were materials actually formulated as drug delivery systems for topical treatments, but were examined to determine if they might display any protective efficacy. In consideration of proprietary issues, results shown below are coded by internal USAMRICD numbers.

In general, samples were applied as a layer of 0.15-mm thickness as described above, then challenged with either 8.0 µl of CEES or HD (in a few cases, a 20-µl challenge was employed as noted) within 15 minutes of application of the candidate topical protectant. In addition, in cases where the candidate topical protectant was aqueous based, the layer was also challenged following a 30-minute drying period after application, to determine if penetration was influenced by the presence of residual water from the formulations.

Test results for the various topical protectants are shown in Table 3. Exposures were terminated at 60 minutes. Breakthrough was assessed as the point at which a visible color change could be noted either by examining the underside of the M8 paper or by a color change visible through the topical protectant layer. In no case did the topical protectant itself cause a color change in the paper that completely masked breakthrough.

CEES results appear to parallel those found for mustard penetration. It may be possible to substitute the less hazardous CEES for mustard in future tests or for testing at laboratories where utilization of chemical agents is not feasible.

Table 3: Breakthrough Times for Topical Protectants Challenged with either HD or CEES[a]

TOPICAL	CEES			HD		
PROTECTANT	TIME (MIN)	N	SD	TIME (MIN)	N	SD
1511	57.0	16	6.3	60+	14	---
1472	0.7	15	0.3	1.3	15	0.7
1510	0.2	16	0.1	0.2	13	0.1
1512	0.1	16	0.1	0.1	14	0.1
1743	0.2	16	0.0	0.9	16	0.6
1744	0.3	16	0.1	0.6	15	0.3
1509	0.42	16	0.6	8.0	16	7.8
1465	50.0	23	13.6	60+	12	---
1466	58.0	40	12.4	60+	14	---
1467	60+	24	---	60+	14	--
1468	0.4	40	0.3	1.1	15	0.7
1469	60+	24	---	60+	16	---
1463	2.0	15	1.0	4.3	14	1.5
1464	1.5	16	0.9	0.7	14	0.5
1536	49.0	16	24.0	60+	12	---
PEG 540	60+[b]	16	---	38.0	30	28
1623	0.70	16	1.1	6.1	15	3.5
1621	3.9	16	1.9	6.5	16	2.2
1622	3.67	16	2.3	11.8	16	4.3
1679	3.37	32	4.07	---	---	---
1689	52.8	24	16.3	56.4	16	11.2
1690	60+	24	---	60+	16	---
1691	60+	24	---	60+	16	---
1692	33.8	24	26.7	57.7	24	9.7

[a]Breakthrough times in minutes for layers challenged with 8 μl (except as noted) CEES or HD. N = number of test assemblies; SD = standard deviation of mean, in minutes. [b]20μl challenge.

Unthickened perfluorinated polyalkyl ethers did not effectively hinder penetration of either CEES or HD. It is of note that the only unthickened oil that showed any indication of increased breakthrough time was ICD #1463, a 3500 cSt high-viscosity oil. Formulations which included polytetrafluoroethylene (PTFE), that is ICD # 1465, 1466, 1467, and 1469 blocked mustard penetration for all test assemblies and blocked CEES penetration either completely or in the majority of cases for 60 minutes. Test topical protectant ICD #1468, a perfluorinated ether thickened with Cab-O-Sil instead of PTFE, gave poor protection against mustard and CEES. This and the result with the unthickened oils indicate that PTFE (and perhaps other thickeners) play a critical role in efficacy of these protectants.

Some difference in breakthrough times was found for layers of aqueous-based formulations that were allowed to dry thoroughly vs only partially prior to challenge with CEES and HD. Table 4 summarizes these results for the aqueous-based topical protectants.

Table 4: Breakthrough Times for Air-Dried vs Non-Dried Layers from Aqueous Based Formulations[a,b,c]

	CEES		Mustard	
Protectants	Wet	Dry	Wet	Dry
1509[d]	*30.0(±21.0)	0.40(±0.56)	*23.0(±11.5)	8.0(±8.0)
1623[d]	*12.5(±15.0)	0.70(±1.10)	*10.7(±12.0)	6.1(±3.5)
1621[d]	3.1(±1.5)	3.9(±2.00)	*19.7(±5.00)	6.5(±2.0)
1622	3.2(±2.2)	3.7(±2.35)	11.4(±8.00)	11.8(±4.0)
1536	49.0(±19.0)	48.9 ±25.0	45.3(±27.0)	60+

[a]Candidate topical protectants challenged immediately following application (wet) or after a 30-min drying period (dry). [b]Time-to-breakthrough in minutes. [c]Numbers in parentheses are standard deviations (in minutes) for mean breakthrough times. [d]Results significantly different from air-dried layers, at the P = 0.05 level.

As may be seen by the data, drying in general decreased the breakthrough time for these topical protectants except in the case of ICD # 1536. The performance against HD of this candidate topical protectant appears to improve with thorough drying of the layer. Aqueous-based topical protectants such as ICD # 1509 and ICD # 1536 were found to be difficult to spread into an even layer. Generally, the aqueous-based formulations wet the M-8 paper, causing it to wrinkle and thus making achievement of a uniform coating very difficult. Even given this caveat, based on preliminary results from animal testing we find that qualitatively the data for the candidate protectants does reflect their in vivo efficacy. This applies to both the aqueous-based and non-aqueous candidate protectants.

CONCLUSIONS

We have developed a screening method which provides useful information in the evaluation of candidate topical protectants for efficacy against mustard and CEES. The method provides data that can be used qualitatively to rank-order formulations for testing in more sensitive *in vitro* or *in vivo* studies. Based on preliminary *in vivo* testing results, compounds that fail to provide significant protection against penetration of HD (or CEES) in this simple test appear to provide lessened protection in animal testing when compared to those topical protectants that fully block penetration. This screening procedure provides more rapid results than those available through animal testing and will allow for elimination of candidates that show no evidence of potential efficacy. Thus, the number of animals required will be significantly reduced. Further, we have identified candidate topical protectants that show great promise as barriers against the chemical agent HD, even at a larger thickness of a 1 mm nominal.

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